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RESEARCH ARTICLE

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Opposite impact of *Methylene tetrahydrofolate reductase C677T* and *Methylene tetrahydrofolate reductase A1298C* gene polymorphisms on systemic inflammation

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Background: Methylenetetrahydrofolate reductase (*MTHFR)* gene polymorphisms have been found to be related with many diseases. Systemic inflammation is now considered as a major predisposition factor for diseases including diabetes mellitus (DM), coronary arterial disease (CAD), stroke, and cancer. This study aimed to investigate whether systemic inflammation is a possible underlying pathogenesis for *MTHFR* gene polymorphism-related disease.

Methods: A total of 292 patients were enrolled, and single nucleotide polymorphisms for *MTHFR C667T* and *A1298C* were genotyped. Systemic inflammation markers, neutrophil-to-lymphocyte ratio (NLR), and platelet-to-lymphocyte ratio (PLR) were collected.

Results: In our study population, *MTHFR 677* variants had significant higher NLR level than *MTHFR 677* wild type (3.77 ± 0.26 vs 3.06 ± 0.18, $P = .028$). Logistic regression analysis showed that *MTHFR 677* variants were significantly associated with increased NLR level. *MTHFR 1298* variants showed the opposite effects which tended to have lower level of NLR (3.21 ± 0.16 vs 3.79 ± 0.34, $P = .087$) and PLR (137.0 ± 4.8 vs 157.7 ± 9.4, $P = .052$) than *MTHFR 1298* wild type. General linear model showed that there was no statistically significant interaction between *MTHFR C667T* and *A1298C* gene polymorphism on NLR or PLR.

Conclusions: This study indicates that *MTHFR C677T* and *MTHFR A1298C* gene polymorphisms have opposite effect on systemic inflammation, and systemic inflammation may contribute to the pathogenesis for diseases associated with *MTHFR C667T* gene polymorphism.

KEYWORDS

gene polymorphisms, inflammation, MTHFR, neutrophil-to-lymphocyte ratio, platelet-to-lymphocyte ratio

1 | INTRODUCTION

MTHFR is one of the key enzymes in folic acid and homocysteine metabolism. It is responsible for the irreversible conversion of 5,10-methylenetetrahydrofolate to 5-methylTHF, which serves as methyl donor for methylation of homocysteine to methionine. Two most common *MTHFR* gene polymorphisms, *C677T* (Ala222-Val, rs1801133) and *A1298C* (Glu429Ala, rs1801131), have been widely investigated. It is believed that this 2 gene polymorphisms affect 30%-50% of general population and are related with increased risks for many diseases. *MTHFR C677T* polymorphism was found to be related with diabetes,[1,2] insulin resistance,[3] risk of coronary artery

disease (CAD), premature CAD, and severity of CAD.[4-6] In a meta-analysis, *C677T* polymorphism was shown to be associated with the risk of myocardial infarction (MI) in African, North American, and elderly populations.[7] Ischemic stroke patients also have higher percentage of *MTHFR C667T* variants.[8,9] Recently, *MTHFR C667T* variants were also found to be related with inflammatory disease such as psoriasis[10] and inflammatory bowel disease (IBD).[11] Both *MTHFR C667T* and *A1298C* variants were shown to be associated with rheumatoid arthritis.[12,13] There are also evidences suggest the association between *MTHFR* gene polymorphism and cancers. Liu et al[14] demonstrated that *MTHFR 677TT* genotype might be associated with an increased lung cancer risk. Meta-analyses supported the association of *MTHFR* C677T polymorphism with cervical cancer,[15] breast cancer,[16] non-Hodgkin lymphoma,[17] oral cancer,[18] and overall risk of carcinogenesis.[19]

The mechanism underlying the association between *MTHFR* gene polymorphisms and diseases is not very clear. Most previous studies focused on elevated homocysteine level and folic acid metabolism. However, previous studies did not consistently support that elevated homocysteine level underlying the pathogenesis of these diseases related to *MTHFR* gene polymorphisms. Most importantly, folic acid supplement therapy, although significantly reduced homocysteine level, still failed to improve *MTHFR* gene polymorphism-related disease risks or disease outcome in most large clinical trials.

Systemic inflammation is known as a key factor in the pathogenesis for not only chronic inflammatory disease such as rheumatoid arthritis (RA), IBD, and psoriasis but also for many other diseases including CAD,[20] diabetes mellitus (DM),[21] and cancer.[22] Patients with diabetes with *MTHFR 677* homozygous mutant (TT) have been shown to have elevated C-reactive protein (CRP) and IL-6 level.[23] So it is reasonable to have the hypothesis that chronic systemic inflammation is one of the underlying pathophysiologies for diseases associated with *MTHFR* gene polymorphism. In this study, we intended to investigate whether *MTHFR* gene polymorphism causes systemic inflammation by measuring 2 systemic inflammation markers, neutrophil-to-lymphocyte ratio (NLR), and platelet-to-lymphocyte ratio (PLR).

2 | METHODS

2.1 | Subjects

A total of 292 patients were included in this study, and a consent form was signed by every patient. This study was approved and monitored by Copernicus Group Institutional Review Boards. This study was in compliance with the Declaration of Helsinki.

2.2 | Genotyping

We used the magnetic bead-based method for concentrating DNA, which was obtained from buccal swab leukocytes using QiangenQiaCube instrument and MagMAXTM DNA Multi-sample kits. *MTHFR* gene polymorphism *C677T* (Ala222-Val, rs1801133) and *A1298C* (Glu429Ala, rs1801131) were genotyped in 292 patients.

2.3 | Study outcome definition

Diabetes mellitus was diagnosed if the fasting plasma glucose concentration was ≥126 mg/dL at 2 different time points or hemoglobin A1c ≥ 6.5%. Hypertension was defined as systolic blood pressure ≥140 mm Hg and/or diastolic blood pressure ≥90 mm Hg or treatment with antihypertensive medication. Coronary artery disease (CAD) is defined as who had a minimum of one angiographically documented coronary artery stenosis >50%.

2.4 | Statistics

SPSS 16 was used for statistical analysis. Data are presented as mean ± standard error, and $P < .05$ was considered to be statistically significant. One-way analysis of variance and Crosstabs distribution with chi-square (χ^2) analysis were performed to evaluate the differences between data. Pairwise linkage disequilibrium was characterized by D' value using the CubeX-online web tool.[24] Binary logistic regression was used to evaluate the association of *MTHFR* gene polymorphism and NLR and PLR. General lineal model (GLM) was used to assess the interaction between *MTHFR 677* and *1298* on NLR and PLR.

3 | RESULTS

3.1 | Baseline characteristics of study population

The characteristics of all patients are list in Table 1. Among the total 292 patients, 129 patients were *MTHFR 677* wild type (*MTHFR 677 CC*) and 163 patients were *MTHFR 677* variants (136 patients for *MTHFR 677 CT* and 27 patients for *MTHFR 677TT*). For *MTHFR 1298*, 117 patients were wild type (*MTHFR 1298AA*) and 175 patients were *MTHFR 1298* variants (147 patients for *MTHFR 1298AC* and 28 patients for *MTHFR 1298CC*). There were no statistical differences in baseline characteristics between wild type and variants for both *MTHFR 677* and *1298*. Previous studies have suggested that patients with *MTHFR 677* variants have higher risk for DM, CAD, and hypertension. Our results did not show statistical differences in DM, CAD, and hypertension risk between *MTHFR 677* and *MTHFR 1298* wild type and variants.

3.2 | *MTHFR* gene polymorphism distribution and linkage disequilibrium between *MTHFR C677T* and *MTHFR A1298C*

The genotype distribution of *MTHFR 677* and *MTHFR 1298* is presented in Table 2. *MTHFR C677T* and *MTHFR A1298C* are at complete linkage disequilibrium (D' = 1) in our study population. Patients with *MTHFR1298CC* only appear in *MTHFR 677CC* patients groups, and patients with *MTHFR 677TT* only appear in *MTHFR 1298 AA* patients group.

TABLE 1 Clinical characteristics of study population

Variable	*MTHFR 677* wild type (n = 129)	*MTHFR 677* variants (n = 163)	*P*	*MTHFR 1298* wild type (n = 117)	*MTHFR 1298* variants (n = 175)	*P*
Gender (men), n (%)	72 (55.8%)	100 (61.3%)	.402	65 (55.6%)	107 (61.1%)	.396
Age, y	71.1 ± 1.2	70.8 ± 1.0	.833	70.6 ± 1.1	71.1 ± 1.0	.731
Neutrophil, K/μL	4.36 ± 0.16	4.74 ± 0.16	.101	4.53 ± 0.17	4.60 ± 0.16	.749
Platelet, K/μL	200.7 ± 5.4	203.6 ± 4.6	.679	207.5 ± 5.5	198.7 ± 4.5	.214
Lymphocyte, K/μL	1.63 ± 0.05	1.61 ± 0.06	.841	1.62 ± 0.07	1.62 ± 0.05	.936
FBG, mg/dL	113.4 ± 2.8	114.4 ± 2.9	.808	117.6 ± 3.9	111.4 ± 2.2	.137
LDL-c, mg/dL	85.1 ± 2.6	85.2 ± 2.4	.981	83.7 ± 2.7	86.1 ± 2.3	.500
Cr, mg/dL	1.17 ± 0.07	1.22 ± 0.07	.620	1.1 ± 0.1	1.2 ± 0.1	.350
eGFR, mL/min/1.73 m^2	64.4 ± 2.0	65.9 ± 2.2	.615	65.0 ± 2.4	65.3 ± 1.9	.914
CAD, n (%)	29 (22.5%)	26 (16.0%)	.176	20 (17.1%)	35 (20.0%)	.647
HTN, n (%)	59 (45.7%)	64 (39.3%)	.284	45 (38.5%)	78 (44.6%)	.334
DM, n (%)	54 (41.9%)	65 (39.9%)	.811	49 (41.9%)	70 (40.0%)	.716

FBG, fasting glucose; LDL-c, LDL-cholesterol; Cr, serum creatinine; eGRF, estimated glomerular filtration rate; CAD, coronary artery disease; HTN, hypertension; DM, diabetes mellitus.

TABLE 2 MTHFR gene polymorphism distribution and linkage disequilibrium between *MTHFR 677* and *MTHFR 1298*

	MTHFR 1298AA	*MTHFR1298AC*	*MTHFR1298CC*	Total patient number
MTHFR 677CC	22	79	28	129
MTHFR 677CT	68	68	0	136
MTHFR 677TT	27	0	0	27
Total patient number	117	147	28	292

3.3 | Opposite effect of *MTHFR 677* and *MTHFR 1298* variants on NLR and PLR

Neutrophil-to-lymphocyte ratio (NLR) and platelet-to-lymphocyte ratio (PLR) are markers for systemic inflammation. Table 3 shows that *MTHFR 677* variants had significant higher NLR level than *MTHFR 677* wild type (3.77 ± 0.26 vs 3.06 ± 0.18, *P* = .028). *MTHFR 1298* variants showed the opposite effect on systemic inflammation. *MTHFR 1298* variants tended to have lower level of NLR (3.21 ± 0.16 vs 3.79 ± 0.34, *P* = .087) and PLR (137.0 ± 4.8 vs 157.7 ± 9.4, *P* = .052) than *MTHFR 1298* wild type, with almost statistically significance.

3.4 | Interactions between *MTHFR 677* and *MTHFR 1298* on NLR and PLR

Previous studies have shown that interactions between MTHFR 677 and *MTHFR 1298* have impact on clinical phenotype and disease risk. To analyze whether there are interactions between *MTHFR 677* genotypes and *MTHFR 1298* genotypes on NLR and PLR, an univariate general linear model was used to test the potential interaction. *MTHFR 677* was grouped with 2 different methods as follow: group 1, *MTHFR 677 CC*, *CT*, and *TT*. Group 2, *MTHFR 677* wild type (CC) and variants (CT and TT). *MTHFR 1298* was also grouped with 2 different methods in the same way. No statistically significant interaction was found between *MTHFR 677* genotypes and *MTHFR 1298* genotypes on NLR or PLR in all 2 grouping methods. So the reduction in NLR and PLR in *MTHFR 1298* variants was not due to its linkage disequilibrium with the *C677T* polymorphism or an interaction between *MTHFR 677* and *MTHFR 1298* genotypes.

3.5 | Associations between *MTHFR 677* and NLR and PLR

Logistic regression model was used to analyze the association between *MTHFR 677* variants and clinical and biochemical outcomes of our patient population. Table 4 shows that *MTHFR 677* variants were associated with increased NLR level significantly. There was no association of *MTHFR 677* variants with PLR, DM, hypertension (HTN), and CAD in our patient population.

We also tested the associations between *MTHFR 1298* variants and NLR or PLR. There was no association between *MTHFR 1298* variants with NLR (OR = 0.985, 95%CI, 0.854-1.137, *P* = .838) or PLR (OR = 0.996, 95%CI, 0.991-1.001, *P* = .161).

4 | DISCUSSION

It is still very controversial that whether hyperhomocysteinemia is a causal factor for *MTHFR* gene polymorphism-related diseases.

TABLE 3 Opposite effect of *MTHFR 677* and *MTHFR 1298* variants on NLR and PLR

Variable	*MTHFR 677* wild type (n = 129)	*MTHFR 677* variants (n = 163)	*P*	*MTHFR 1298* wild type (n = 117)	*MTHFR 1298* variants (n = 175)	*P*
NLR	3.06 ± 0.18	3.77 ± 0.26	.028*	3.79 ± 0.34	3.21 ± 0.16	.087
PLR	139.5 ± 7.4	150.4 ± 6.4	.264	157.7 ± 9.4	137.0 ± 4.8	.052

NLR, neutrophil-to-lymphocyte ratio; PLR, platelet-to-lymphocyte ratio.
*Statistical significance

Tutuncu et al[1] suggested that in their study population, patients with type 2 diabetes patients had similar fasting plasma homocysteine levels with that of age- and sex-matched healthy people. On the contrary, *MTHFR C677T* variants were still found to be more frequent in patients with diabetes. The results from Husemoen et al[25] also did not support a relationship between homocysteine and CAD. However, they suggested that patients with the *MTHFR 677 TT* genotype had a higher risk of CAD (HR 1.38, 95%CI 1.11-1.71), and this association was not modified by folate status. Many other large clinical trials also showed that folic acid combined with vitamin B (6) or B (12) treatment significantly reduced plasma homocysteine level but did not affect the risk of cardiovascular disease,[26,27] stroke,[27] and no significant effect on the risk of new-onset diabetes DM[28] or on HbA1c levels.[29] Folic acid supplement therapy also had no significant effect on the incidence of cancer of the large intestine, prostate, lung, breast, or any other specific site and also no effect on overall cancer incidence.[30]

Systemic inflammation is now considered as a major predisposition factor for many diseases including DM, CAD, stroke, and cancer. Studies have uncovered an inflammatory process in beta-cell islets of patients with type 2 diabetes characterized by the presence of cytokines, immune cells, beta-cell apoptosis.[31] Insulin resistance is also linked to inflammatory cytokine stimulation.[32] Atherosclerosis is now considered as a chronic vascular inflammation. Macrophages, foam cells, lymphocytes, and other inflammatory cells are found in the intimal atherosclerotic lesions. Inflammatory cytokines such as tumor necrosis factor alpha (TNF-α), interleukin-1 (IL-1), interleukin-6 (IL-6), and monocyte chemoattractant protein-1 (MCP-1) also play a pivotal role in all stages of atherosclerosis. Many cancers arise from sites of infection, chronic irritation, and inflammation. Tumor microenvironment, which is an indispensable participant in the neoplastic process, is largely orchestrated by inflammatory cells.[33] NLR and PLR are markers of systemic inflammation. Elevated NLR and/or PLR levels were shown to be related with the risks for many diseases or disease outcome. For example, elevated NLR and PLR were shown to associate with DM,[34,35] complications of DM,[36-39] prevalence, severity, and prognosis of CAD,[40-43] severity of lower extremity peripheral artery disease,[44] different types of cancer and cancer prognosis,[45-48] and also inflammatory disease such as IBD,[49] rheumatoid arthritis.[50] Our results suggest for the first time that *MTHFR 677* variants are associated with elevated NLR level, and systemic inflammation is possible pathogenesis for diseases associated with *C677T MTHFR* polymorphism. The mechanism for *MTHFR C677T* polymorphism-caused systemic inflammation is not very clear. Hyperhomocysteinemia and DNA hypomethylation in *MTHFR C677T* polymorphism are 2 possible factors that cause systemic inflammation in *MTHFR* gene variants patients. Homocysteine was shown to be significantly associated with serum C4, CRP, and IgM level,[51] and DNA hypomethylation of some of genes was also shown to be an important factor in inflammation.[52] So it is possible that hyperhomocysteinemia and/or DNA hypomethylation cause systemic inflammation in *MTHFR C677T* variants patients, and systemic inflammation becomes a more direct and more relevant pathogenesis for *MTHFR C677T* polymorphism-related disease.

TABLE 4 Logistic regression model for *MTHFR 677* variants

	OR	95%CI	*P*
NLR	1.183	1.003-1.397	.047*
PLR	0.999	0.994-1.004	.646
FBG, mg/dL	1.002	0.993-1.011	.645
LDL-c, mg/dL	0.996	0.987-1.006	.442
Cr, mg/dL	0.895	0.567-1.411	.632
eGFR, mL/min/1.73 m^2	1.004	0.990-1.018	.569
CAD (%)	1.325	0.672-2.613	.417
HTN (%)	1.192	0.683-2.083	.536
DM (%)	1.166	0.624-2.177	.631

NLR, neutrophil-to-lymphocyte ratio; PLR, platelet-to-lymphocyte ratio; FBG, fasting glucose; LDL-c, LDL-cholesterol; Cr, serum creatinine; eGRF, estimated glomerular filtration rate; CAD, coronary artery disease; HTN, hypertension; DM, diabetes mellitus.
*Statistical significance

Another intriguing finding of this study is that *MTHFR 1298* variants had opposite effects on systemic inflammation compared with *MTHFR677* variants. Both NLR and PLR were tend to be higher in patients with *MTHFR 1298* variants compared with patients with *MTHFR 1298* wild-type genotype. Previous clinical studies suggested that *MTHFR1298* variants had different association with diseases compared with *MTHFR677* variants. For example, Alizadeh et al[7] suggested that the *A1298C* polymorphism was not significantly associated with MI risk. Unlike *A1298C* polymorphism, *C677T* polymorphism was associated with risk of MI. Yuan et al[12] indicated an association between the *MTHFR C677T* polymorphism and the risk of RA in Caucasian population. However, there is no evidence of significant association between *A1298C* polymorphism and RA risk in Caucasian population. Basic researches also showed that *MTHFR 677* and *1298* polymorphism have different and even opposite effect on cell and metabolism. *MTHFR A1298C* variants have much less effect on the stability of MTHFR protein, level of homocysteine, and DNA methylation than *MTHFR 677* variants. Parle-McDermott et al[53] reported that the *MTHFR 1298*

variants and *677* variants had opposite associations with red cell folate levels. Our results add more evidences that *MTHFR 677* variants and *1298* variants may have different and even opposite effect on disease risk or clinical phenotype.

This study also showed that *MTHFR C677T* and *MTHFR A1298C* were at complete linkage disequilibrium (D' = 1) in our study population. Fan et al[54] showed that *MTHFR C677T* and *MTHFR A1298C* were at high linkage disequilibrium among Chinese, and the percentage of patients with *677CT/1298CC*(0.4%), *677TT/1298AC* (0.7%), and *677TT/1298CC* (0.1%) were extremely low. As most patients with *MTHFR1298CC* (homozygous mutant) are *MTHFR 677CC* (wild type) patients and most patients with *MTHFR677TT* (homozygous mutant) are *MTHFR1298AA* (wild type) patients, LD should be taken into consideration when investigate effect of this 2 gene polymorphism on disease risk and clinical phenotype. For example, some previous studies showed that *MTHFR 1298 CC* had protective effect on methorexate-related adverse effects[55] or significantly lower risk of acute lymphocytic leukemia[56]; however, all these studies did not investigate whether the effect of *1298CC* is due to its high LD with *MTHFR 677* wild-type genotype. Our results showed that there was no statistically significant interaction between *MTHFR 677* genotypes and *MTHFR 1298* genotypes on NLR or PLR. So the reduction in NLR and PLR in *MTHFR 1298* variants patients is not due to its linkage disequilibrium with *MTHFR 677* or an interaction between *MTHFR 677* and *MTHFR 1298*.

In summary, this study indicates that *MTHFR* gene polymorphisms have effect on systemic inflammation. *MTHFR 677* variants have significantly higher NLR level than *MTHFR 677* wild type while *MTHFR 1298* variants showed the opposite effect on systemic inflammation. *MTHFR 1298* variants tend to have lower level of NLR and PLR than *MTHFR 1298* wild type. Systemic inflammation may contribute to the pathogenesis for diseases associated with *MTHFR C667T* gene polymorphism.

AUTHOR CONTRIBUTIONS

The study was conceived and designed by Koroush Khalighi, Gang Cheng provided statistical and analytical support. Seyedabbas Mirabbasi, Bahar Khalighi, Yin Wu, Wuqiang Fan were responsible for sample collection. Koroush Khalighi and Gang Cheng wrote the study.

The authors alone are responsible for the content and writing of the study.

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